Annals of Clinical and Analytical Medicine
Original Research

Investigation of the change in the level of pro-inflammatory, anti-inflammatory cytokine and total antioxidant in obese patients

Change in the level of inflammatory cytokine and total antioxidant in obese patients

Tugba Elgun[1], Enver Ciraci[2], Alper Ozturk[3]
[1] Department of Medical Biology, Faculty of Medicine
[2] Department of Biochemistry, Faculty of Pharmacy
[3] Department of General Surgery, Faculty of Medicine, Biruni University, Istanbul, Turkey

Abstract

Aim: Obesity and its complications are an increasing problem in developed countries. In 2011, around 500 million people worldwide suffered from obesity (body mass index (BMI) >30 kg/m^2) and this figure is projected to at least double by 2030. The aim of this study was to investigate the changes in pro-inflammatory, anti-inflammatory cytokines and total antioxidant levels (TAC) in obese patients.

Material and Methods: In our study, IL-6, which is proinflammatory inteukin (IL), and anti-inflammatory IL-33 and total antioxidant levels were determined in 56 obese patients (BMI>30 kg/m^2) and 50 control groups (18.5 <BMI). It is aimed to evaluate <24.9 kg/m2). IL-6 and IL-33 levels were investigated by ELISA (Enzyme-Linked Immuno Sorbent Assay) method.

Results: IL-6 and IL-33 levels were found to be significantly higher in the obese patient group compared to the control group ($p<0.05$). The total antioxidant level was found to be significantly lower in the obese patient group compared to the control group ($p<0.001$).

Discussion: Morphological and functional changes that occur in AD during the development of obesity can cause local and systemic inflammation. Released cytokines, fatty acids, free radicals and other factors affect the immunological activity of all tissues and cells. Chronic inflammation associated with obesity leads to many health complications such as cardiovascular and liver diseases.

Keywords

IL-6, IL-33, Metabolic Syndrome, Inflammation

DOI: 10.4328/ACAM.21813 Received: 2023-07-08 Accepted: 2023-08-14 Published Online: 2023-08-18 Printed: 2023-10-15 Ann Clin Anal Med 2023;14(Suppl 3):S230-233
Corresponding Author: Tugba Elgun, Department of Medical Biology, Faculty of Medicine, Biruni University, Zeytinburnu, Istanbul, Turkey.
E-mail: telgun@biruni.edu.tr P: +90 505 316 37 63
Corresponding Author ORCID ID: https://orcid.org/0000-0002-1311-6892
This study was approved by the Ethics Committee of Biruni University (Date: 2023-03-29, No: 2023/79-33)

Introduction

The prevalence of obesity, which has become a global epidemic, has tripled worldwide since 1975. A body mass index (BMI, weight in kilograms divided by height in meters squared) equal to or greater than 30 is defined as obesity, which is associated with increased morbidity and mortality [1]. Obesity is an important risk factor for chronic diseases such as cardiovascular disease, diabetes mellitus (DM), chronic kidney disease, cancers, musculoskeletal disorders and obstructive sleep apnea. More than 80% of obese individuals are prone to develop insulin resistance during their lifetime. Enlarged adipocytes release free fatty acids (FFAs), lipopolysaccharide, reactive oxygen species and proinflammatory cytokines, causing lipotoxicity in non-adipose organs including the liver, muscle and pancreas. Disorganized organelles contribute to systemic inflammation that causes insulin resistance by inhibiting insulin signaling pathways [2].

Inflammation is a sequential cascade of events designed to maintain tissue and organ homeostasis. Timely release of mediators and expression of receptors are essential for program completion and restoration of tissues to their original state [3]. In addition, inflammation is a protective tissue response to injury or destruction of tissues that serves to destroy or dilute both the damaging agent and injured tissues [4].

Leptin, interleukin 6 (IL-6), tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), and resistance [5]. Obesity, a feature of metabolic syndrome, is associated with chronic inflammation in obese subjects [6].

Interleukin 6 is a cytokine that mediates inflammatory responses and is produced by many different cell types, including immune cells and adipose tissue [7]. Unlike other cytokines, IL-6 is unusual in that its main effects occur at sites different from its source and result from its circulating concentrations. For this reason, it is called an endocrine cytokine [8]. Adipose tissue produces various pro-inflammatory and anti-inflammatory factors, including the adipokines leptin, adiponectin, and resistin, as well as cytokines and chemokines such as TNF-α, IL-6, and MCP-1 [9].

Positive relationships between different measures of obesity and plasma IL-6 levels have been described [10]. It has been calculated that one-third of the total circulating concentrations of IL-6 originate from adipose tissue [11].

Proinflammatory cytokines overexpressed in obesity are considered to be the link between obesity and inflammation [12]. Adipose tissue responds to the stimulation of extra nutrients through hyperplasia and hypertrophy of adipocytes. The nature of adipose tissue is heterogeneous, including endothelium, immune cells, and adipocytes [13]. With progressive adipocyte growth and obesity, the blood supply to adipocytes may be reduced, leading to hypoxia.

Adipose tissue (AD) is composed of various cell types, including adipocytes, preadipocytes, immature adipocyte precursors, endothelial cells, and immune cells. Three different types of adipocytes found in mammals are white adipocytes, brown adipocytes, and beige adipocytes. White adipocytes are the main source of storage of excess energy. In contrast, brown adipocytes can convert energy into heat. When abnormal or excess energy is stored in adipose tissue, expansion of adipose tissue begins from established tissue precursors or existing adipocytes to form new (hyperplasia) or enlarged adipocytes (hypertrophy). Adipocytes function as endocrine regulators to modulate energy expenditure and systemic health. They secrete a variety of hormones, proteins, and peptides known as adipocyte-derived adipokines and are involved in lipid metabolism, insulin sensitivity, blood pressure regulation, and inflammation [14]. For example, high levels of the proinflammatory markers C-reactive protein (CRP) and IL-6 have been found in obese individuals and represent risk factors for the development of type 2 DM. In addition, other adipokines such as leptin, resistin, RBP4 and TNF-α also have proinflammatory effects and affect cardiovascular functions [15].

In obesity, inflammatory immune cell accumulation in the adipose microenvironment is critical for causing insulin resistance and metabolic dysfunction. In obese individuals, the number of macrophages is increased and they participate in inflammatory pathways. Macrophage infiltration increases in adipose tissues that transition to a proinflammatory microenvironment [16]. In obesity, adipocytes become hypertrophic and disorganized, resulting in inflammatory changes in adipose tissue with the secretion of interferon-γ (IFN-γ) and TNF-α [17]. The adipose microenvironment is disrupted and enriched in M1-polarized ATMs, CD8+ T cells, type 1 T helper (Th1) cells, Th17 cells, and neutrophils [18]. Inflammatory cytokines or chemokines such as IL-6, IL-1β, IL-18, MCP-1, IL-8, and CXCL5 were higher in obese subjects and needed to activate or recruit immune cells.

Material and Methods

Between March 2023 and June 2023, obese patients between the ages of 18 and 40 who were followed up routinely in Biruni University Hospital, Internal Medicine and General Surgery outpatient clinics were included in the study. A total of 106 volunteers were included in the study. There were two patient groups: patients diagnosed with obesity (n=56) and non-obese controls (n=50).

Anthropometric measurements (BMI) and demographic data (age, height and weight) of the individuals included in the study were examined. Venous blood samples were collected from all subjects. Blood samples (serum) were collected in tubes without anticoagulants, centrifuged at 3,000 rpm for 10 minutes, and stored at -80°C until analysis.

IL-33, IL-6 and total antioxidant capacity (TAC) determinations in patients were evaluated by ELISA (Enzyme-Linked Immuno Sorbent Assay) method. Reagents in ELISA kits (BT-LAB, Shanghai Korain Biotech) were prepared and studied in accordance with the procedure. IL-33 (Cat. No: E0044Hu), IL-6 (Cat. No: E0090Hu) and total antioxidant capacity assays (Cat. No: E4350Hu). The calibration standard curve graph was drawn from the absorbance values obtained as a result of the study. IL-33, IL-6 and total antioxidant concentrations of patient serum samples were calculated using the calibration standard curve graph formula.

Prism 9.1.1 (GraphPad Software, La Jolla, CA) was used for all statistical analyses and significance was noted when $P < 0.05$. Differences between the groups were compared by the Mann-Whitney U test. Relationships between variables were tested by Spearman's rank correlations. A two-way ANOVA test was used

to compare the differences in cytokine and TAC concentrations.

Ethical Approval

Ethics Committee approval for the study was obtained. This study was approved by the Ethics Committee of Biruni University (Date: 2023-03-29, No: 2023/79-33).

Results

Demographic characteristics, anthropometric measurements and biochemical parameters of the study subjects are presented in Table I. Obese and control groups had similar maternal age (34 ± 2.1 vs.35 ± 2.3 years, p>0.05). The age of the obese patients ranged from 27 to 42; the age of the control subjects ranged from 25 to 41 years. The BMI in the obese patient group was significantly higher than in the control group (30.41 ± 3.21 vs. 21.23 ± 3.42 kg/m2, p<0.05). CRP (mg/L) in the obese patient group was significantly higher than in the control group (10.96 ± 2.06 vs. 3.61 ± 0.72, p<0.001). The level of triglycerides (mg/dl) was found to be significantly higher in the obese patient group (172.13 ± 1.84) compared to the control group (101.21 ± 1.48, p<0.05). The level of Total cholesterol (mg/ dl) was found to be significantly higher in the obese patient group (224.76 ± 2.41) compared to the control group (97.57 ± 2.84, p<0.001). HDL-cholesterol (mg/dl) levels were found to be significantly higher in the obese patient group (41.62 ± 1.39) compared to the control group (55.98± 1.5, p<0.05) (Table 1).

Levels of IL-6, IL-33 cytokines and TAC in patients with obesity and controls

IL-6 concentration levels in the obese patient group (1.76 ± 0.44) were determined to be higher than in the control group (0.79 ± 0.3, p<0.05). IL-33 concentration levels in the obese patient group (1.05 ± 0.39) were determined to be higher than in the control group (0.67 ± 0.27, p<0.05). TAC in the control group (9.26 ±1.58) was determined to be significantly higher than in the obese patient group (2.8 ± 0.46, p<0.001) (Table 2).

Table 1. Clinical characteristics of the study participants.

Variable	Obese Patient Group (m±SD)	Control Group (m±SD)	P value
Age (year)	34 ± 2.1 (27-42)	35 ± 2.3 (25-41)	>0.05
BMI (kg/m²)	30.41 ± 3.21	21.23 ± 3.42	<0.05
CRP (mg/L)	10.96 ± 2.06	3.61 ± 0.72	<0.001
Triglycerides (mg/dl)	172.13 ± 1.84	101.21 ± 1.48	<0.05
Total cholesterol (mg/dl)	224.76 ± 2.41	97.57 ± 2.84	<0.001
HDL-cholesterol (mg/dl)	41.62 ± 1.39	55.98± 1.5	<0.05

HDL: High-density lipoprotein; LDL: Low-density lipoprotein; CRP: C-Reaktif Protein; BMI: Body mass index.

Table 2. Serum levels of cytokines and TAC of patients with and without obesity.

Variable	Obese Patient Group (±ss)	Control Group (±ss)	P value
IL-6 (pg/ml)	1.76 ± 0.44	0.79 ± 0.3	<0.05
IL-33 (pg/ml)	1.05± 0.39	0.67 ± 0.27	<0.05
TAC (ng/ml)	2.8 ± 0.46	9.26 ±1.58	<0.001

IL: Interleukin; TAC: Total Antioxidant Levels

Discussion

In our study, in 56 obese patients and 50 controls, IL-6 as pro-inflammatory interleukin, IL-33 and TAC as anti-inflammatory interleukin were evaluated.

Illán-Gómez et al. [19] evaluated inflammatory mediators in morbidly obese patients after undergoing bariatric surgery, and they found that IL-6 levels decreased significantly and correlated with BMI after 12 months of surgery (r = 0.53, p < 0.001) and IL- 6 was associated with high CRP levels. Arnardottir et al. [20], in their study evaluating the interaction between obstructive sleep apnea and obesity in IL-6 individuals aged +50 years, the patients were divided into three groups according to BMI (< 30, 30-35 and ≥ 35 kg/m2) and their IL-6 levels were 1.3, 1.3, 1.6 and 2.2 pg/ml showed the relationship between BMI and IL-6.

Fontana et al. [21], in a study where they tested the level of IL-6 and other inflammatory markers between the portal vein and peripheral artery among 25 morbidly obese patients who had undergone gastric bypass surgery in St. Louis, Missouri, they suggested that visceral fat promotes systemic inflammation by secreting inflammatory adipokines into the portal circulation that drains visceral fat. In the study, in which the mean plasma IL-6 concentration was shown to be approximately 50% higher in the portal vein than in the peripheral artery (p = 0.007), portal vein IL-6 concentration was reported to be directly correlated with systemic CRP concentrations (r = 0.544, p = 0.005).

Wannamethee et al. [22], in their study investigating the relationship of IL-6 with metabolic abnormalities in non-diabetic elderly British individuals, showed that, after adjusting for age, an increase in both BMI and waist circumference increased IL-6 levels. IL-6 averages were 2.23, 2.39, and 2.61 pg/ml and were associated with BMI, respectively (< 25.08, 25.08–27.79, and ≥ 27.8 kg/m2). In our study, IL-6 levels were found to be significantly higher in the obese patient group (1.76 ± 0.44 pg/ml) compared to the control group (0.79 ± 0.3 pg/ml) (p<0.05).

In some studies based on obese mouse models, it has been suggested that IL-33 can reduce adiposity, induce anti-inflammatory effects in adipose tissue, and improve glucose and lipid metabolism (28, 29, 30). However, there are no data showing a specific correlation between circulating IL-33 level and human obesity and related metabolic disorders. In our study, we tried to show the correlation between human obesity and its associated metabolic disorders. In our study, IL-33 levels were found to be significantly higher in the obese patient group compared to the control group. (p<0.05).

Mahlakõiv et al. [24] found that adipose-derived IL-33 is upregulated in response to a short-term high-fat diet, promoting Th2 immune response and inducing anti-inflammatory response to maintain tissue homeostasis; this suggests that adipose tissue may stabilize local inflammatory responses.

The pathophysiological characteristics of IL-33 may depend on its secreting cell and its transient expression, as IL-33 can be secreted in an autocrine or endocrine fashion by a variety of cells or tissues. Thus, the correlation between the serum level of IL-33 and its tissue expression in different depots may be more complex. Most previous studies have shown that exogenous IL-33 instead of endogenous IL-33 can regulate glucolipid metabolism and inflammation in obesity [25]. The question of whether endogenous IL-33 can fully maintain the

immune balance of obese adipose tissue is controversial and more research is needed.

Conclusion

Morphological and functional changes that occur in AD during the development of obesity may cause local and systemic inflammation. Released cytokines, fatty acids, free radicals and other factors affect the immunological activity of all tissues and cells. Chronic inflammation associated with obesity leads to many health complications such as cardiovascular and liver diseases. There is a need for a larger sample size and multidisciplinary study with more parameters in this area.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

Funding: *None*

Conflict of interest

The authors declare no conflict of interest.

References

1. Smith KB, Smith MS. Obesity Statistics. Prim Care. 2016;43(1):121-35.
2. Ahmed B, Sultana R, Greene MW. Adipose tissue and insulin resistance in obese. Biomed Pharmacother. 2021;137:111315. DOI: 10.1016/j.biopha.2021.111315.
3. Panigrahy D, Gilligan MM, Serhan CN, Kashfi K. Resolution of inflammation: An organizing principle in biology and medicine. Pharmacol Ther. 2021;227:107879.
4. Peker G, Peker DM, Köseoğlu R, Gül O. Do body composition, hemogram and lipids differ between obese women with and without gonarthrosis? Ann Clin Anal Med. 2023;14(5):399-403.
5. Lafontan M. Fat cells: afferent and efferent messages define new approaches to treat obesity. Annu Rev Pharmacol Toxicol. 2004;45:119-46.
6. Stępień M, Stępień A, Wlazeł RN, Paradowski M, Banach M, Rysz J. Obesity indices and inflammatory markers in obese non-diabetic normo- and hypertensive patients: a comparative pilot study. Lipids Health Dis. 2014;13:29.
7. Ciraci E, Sahin S, Herkiloglu E D, Ahmad S, Unal T, Tetik S. The role of expressed T-cells cytokines mRNAs from endometrial tissue in patients with unexplained infertility. 2023;60(05):406-13.
8. Ghanemi A, St-Amand J. Interleukin-6 as a metabolic hormone. Cytokine. 2018;112:132-6.
9. Jackson VM, Breen DM, Fortin JP, Liou A, Kuzmiski JB, Loomis AK, et al. Latest approaches for the treatment of obesity. Expert Opin Drug Discov. 2015;10(8):825-39.
10. Kacel EL, Kirsch JL, Sannes TS, Patidar S, Postupack R, Jensen S, et al. Interleukin-6 and body mass index, tobacco use, and sleep in gynecologic cancers. Health Psychol. 2019;38(10):866-77.
11. Villarroya F, Cereijo R, Gavaldà-Navarro A, Villarroya J, Giralt M. Inflammation of brown/beige adipose tissues in obesity and metabolic disease. J Intern Med. 2018;284(5):492-504.
12. Hotamisligil GS. Inflammation and metabolic disorders. Nature. 2006;444(7121):860–7.
13. Blaszczak AM, Jalilvand A, Hsueh WA. Adipocytes, Innate Immunity and Obesity: A Mini-Review. Front Immunol. 2021;12:650768.
14. Taylor EB. The complex role of adipokines in obesity, inflammation, and autoimmunity. Clin Sci (Lond). 2021;135(6):731-52.
15. Cabre A, Lazaro I, Girona J, Manzanares J, Marimon F, Plana N, et al. Retinol-binding protein 4 as a plasma biomarker of renal dysfunction and cardiovascular disease in type 2 diabetes. J Intern Med. 2007;262(4):496-503.
16. Wentworth JM, Naselli G, Brown WA, Doyle L, Phipson B, Smyth GK, et al. Pro-inflammatory CD11c+CD206+ adipose tissue macrophages are associated with insulin resistance in human obesity. Diabetes. 2010;59(7):1648-56.
17. Engin AB, Engin A. The Interactions Between Kynurenine, Folate, Methionine and Pteridine Pathways in Obesity. Adv Exp Med Biol. 2017;960:511-27.
18. Morris DL, Cho KW, Delproposto JL, Oatmen KE, Geletka LM, Martinez-Santibanez G, et al. Adipose Tissue Macrophages Function As Antigen-Presenting Cells and Regulate Adipose Tissue CD4+ T Cells in Mice. Diabetes. 2013;62(8):2762–72.
19. Illán-Gómez F, Gonzálvez-Ortega M, Orea-Soler I, Alcaraz-Tafalla MS, Aragón-Alonso A, Pascual-Díaz M, et al. Obesity and inflammation: change in adiponectin, C-reactive protein, tumour necrosis factor-alpha and interleukin-6 after bariatric surgery. Obes Surg. 2012;22(6):950-5.
20. Arnardottir ES, Maislin G, Schwab RJ, Staley B, Benediktsdottir B, Olafsson I, et al. The interaction of obstructive sleep apnea and obesity on the inflammatory markers C-reactive protein and interleukin-6: the Icelandic Sleep Apnea Cohort. Sleep. 2012;35(7):921-32.
21. Fontana L, Eagon JC, Trujillo ME, Scherer PE, Klein S. Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. Diabetes. 2007;56(4):1010-3.
22. Wannamethee SG, Whincup PH, Rumley A, Lowe G. Inter-relationships of interleukin-6, cardiovascular risk factors and the metabolic syndrome among older men. J Thromb Haemost. 2007;5(8):1637-43.
23. Brestoff JR, Kim BS, Saenz SA, Stine RR, Monticelli LA, Sonnenberg GF, et al. Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity. Nature. 2015;519(7542):242-6.
24. Mahlakõiv T, Flamar AL, Johnston LK, Moriyama S, Putzel GG, Bryce PJ, et al. Stromal cells maintain immune cell homeostasis in adipose tissue via production of interleukin-33. Sci Immunol. 2019;4(35):eaax0416. DOI: 10.1126/sciimmunol.aax0416.
25. Miller AM, Asquith DL, Hueber AJ, Anderson LA, Holmes WM, McKenzie AN, et al. Interleukin-33 induces protective effects in adipose tissue inflammation during obesity in mice. Circ Res. 2010;107(5):650-8.

How to cite this article:
Tugba Elgun, Enver Ciraci, Alper Ozturk. Investigation of the change in the level of pro-inflammatory, anti-inflammatory cytokine and total antioxidant in obese patients. Ann Clin Anal Med 2023;14(Suppl 3):S230-233

This study was approved by the Ethics Committee of Biruni University (Date: 2023-03-29, No: 2023/79-33)